Development of NIST SRM 2881

An Absolute Molecular Mass Distribution Polymer Standard

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Goals and Rationales

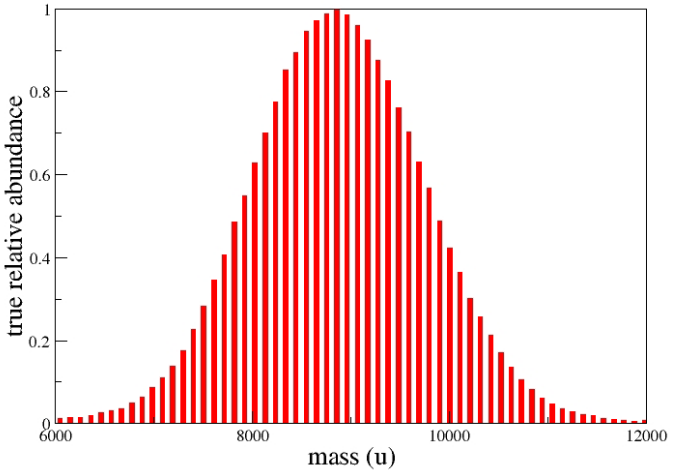
- Current Standard Reference Materials (SRM) give only absolute *moments* of the molecular mass distribution (MMD)
- *Absolute* here means direct measurement of a physical property without reference to another polymer
- Examples: light scattering for M_w; osmometry for M_n
- We wished make an absolute certification of an *entire* MMD
- This SRM would be useful in mass spectrometry and chromatography
- It would also teach us a lot about quantitative MALDI-TOF MS
- Provides a method to develop SRMs of any (proprietary) polymer
- Key: Determine type A (“random”) and type B (“systematic”) uncertainties

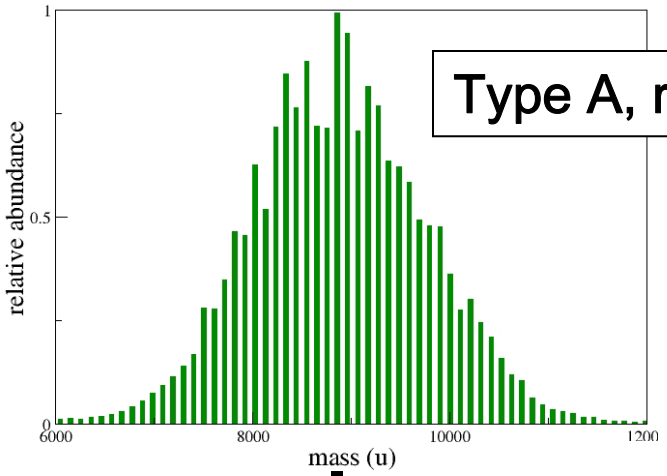
Type A ("Random") Uncertainty

- Obtained by Repeat Measurements
- Mass Axis: relatively small uncertainty
  - Least important MMD determination
  - (Very important for species identification!)
- Signal Axis: larger uncertainty than mass axis
  - Most important axis for MMD measurement
  - Relative peak area (not absolute) is the measurand

Type B ("Systematic") Uncertainty

- Obtained through study of the method itself
- Mass Axis: three calibration strategies
  - Biopolymers (i.e. secondary standards)
  - Calibration on polymer repeat unit mass
  - Voltage, time, distance (absolute, accurate but not precise)
- Signal axis: the most difficult aspect of the problem
  - Complications are associated with polydispersity
    - Desorption probability
    - Ionization probability
    - Detection efficiency
    - Sample preparation

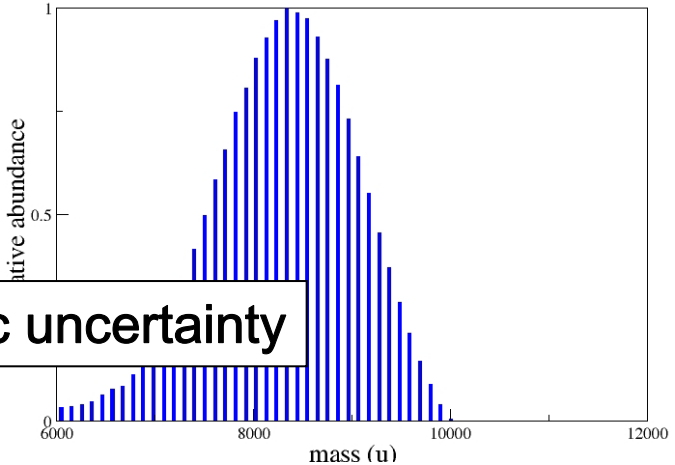


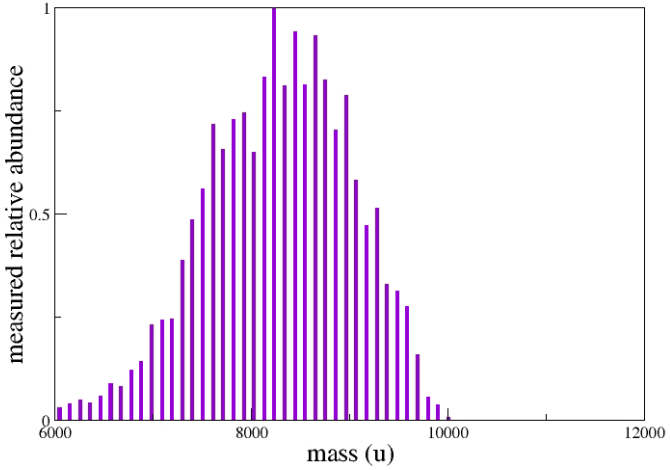


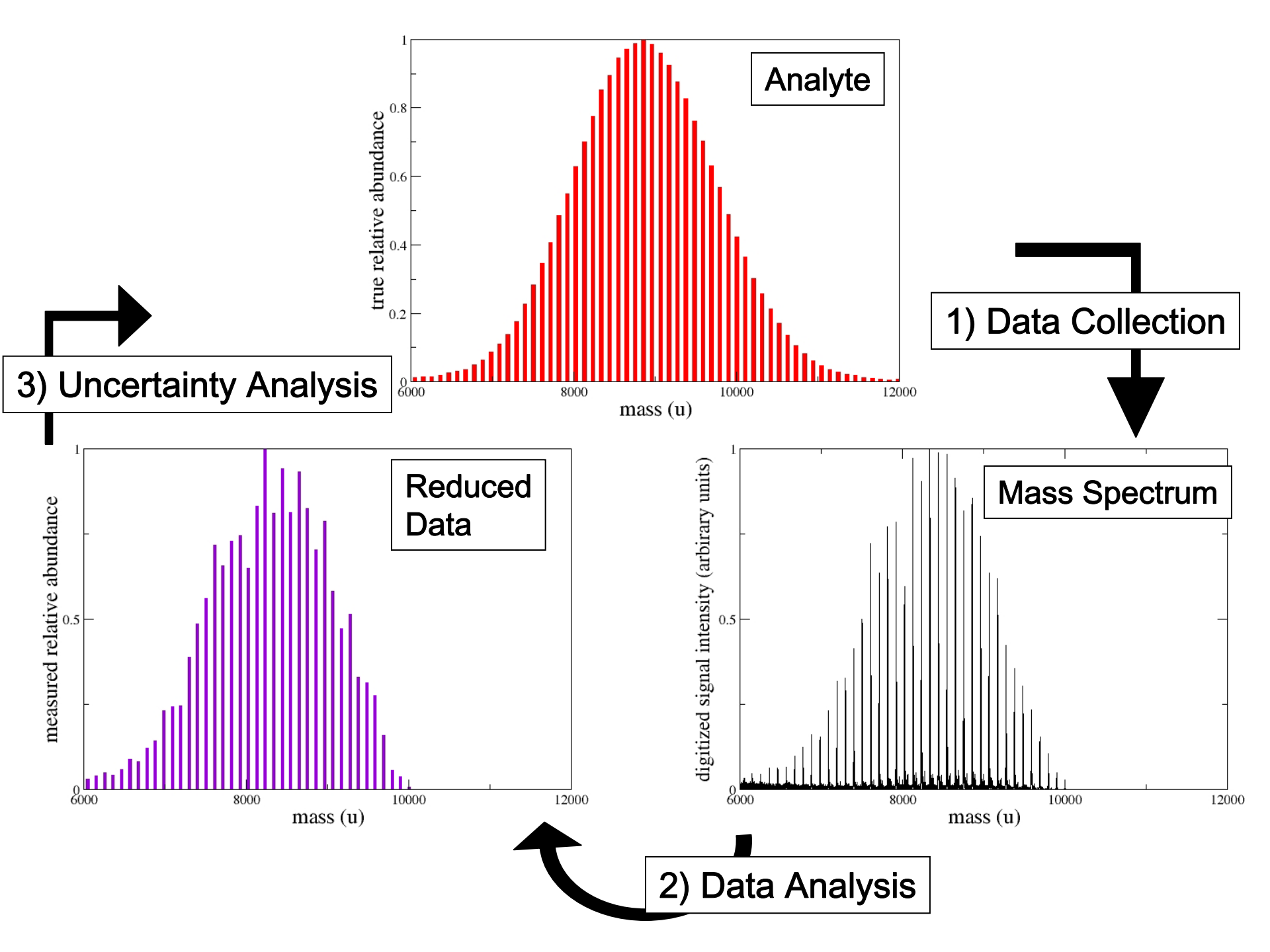
Type A, random uncertainty

+

Type B, systematic uncertainty

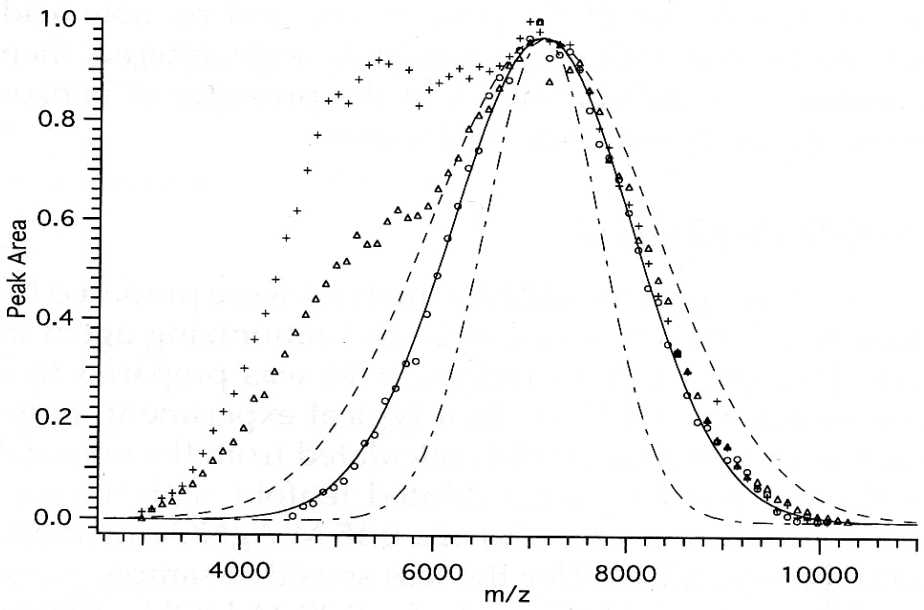





Analyte
true relative abundance
mass (u)
1) Data Collection
Mass Spectrum
digitized signal intensity (arbirary units)
mass (u)
2) Data Analysis
Reduced Data
measured relative abundance
mass (u)
3) Uncertainty Analysis

An Early Demonstration of Quantitation

Liang Li, University of Alberta

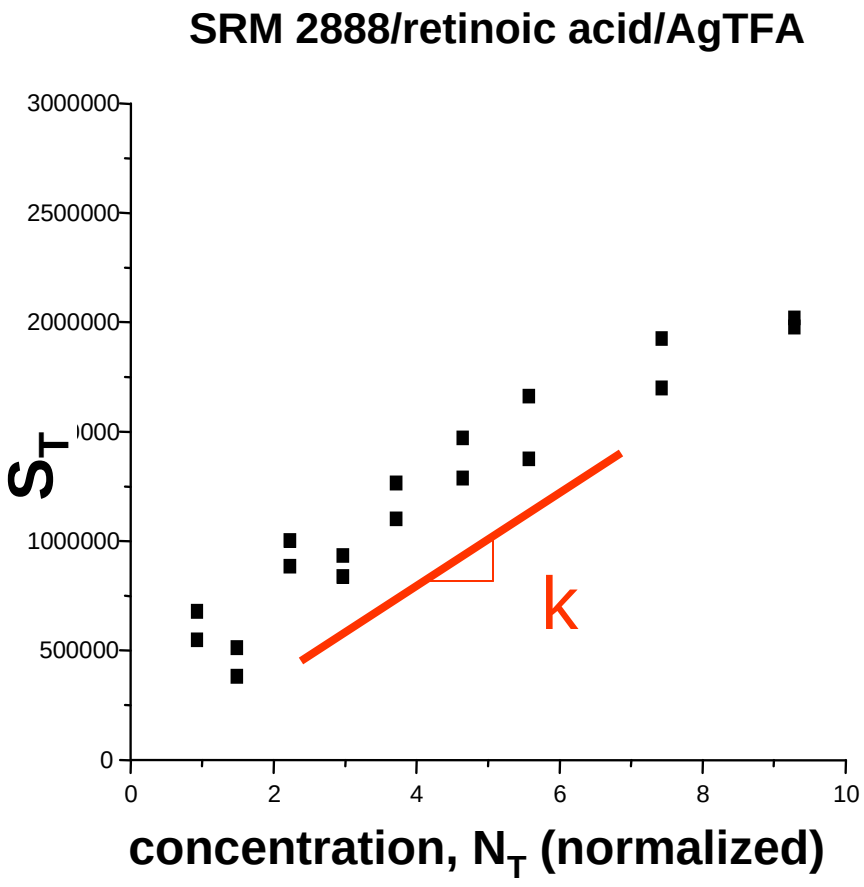


- **Low polydispersity polystyrene mixed in various mole fractions**
- **Demonstrated that polymers with close M_n show additivity of curves**
- **M_n and M_w are correct to within a few percent**
- **Shows $S_i = k\, n_i$ where**
  S_i is peak area
  n_i is number of oligomers
- **Or more generally:**

$$S_T = \Sigma\, S_i = kN_T$$

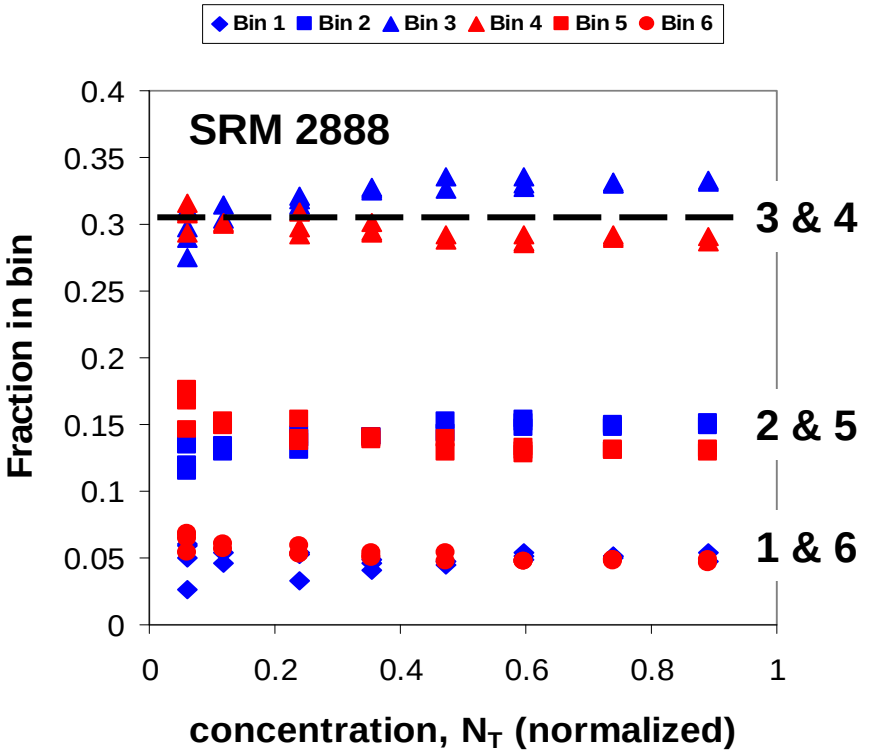
H. Zhu, T. Yalcin, L. Li
J. Am. Soc. Mass Spectrom. 9 (1998) 275

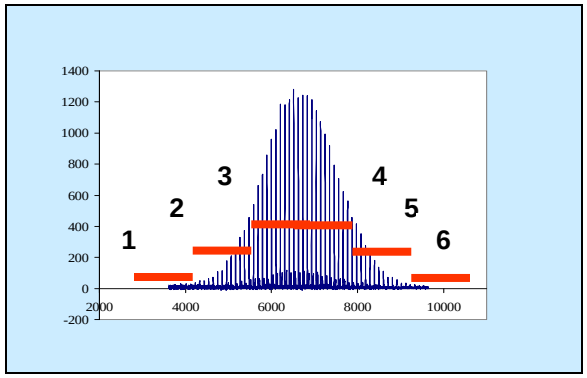
Concentration Independence: Total Signal Intensity



- **$S_T = \Sigma S_i = kN_T$ is a statement that total signal intensity is directly proportional to polymer concentration in the MALDI mixture**
- **This has been demonstrated by several groups for different polymers (e.g. Owens)**
- **There is a region of linearity for many polymers studied**

Concentration Independence: Relative Signal Intensity by Mass





- **Apply this to concentration *within* a distribution**
- **Lines should be horizontal for true mass independence**
- **Regions of non-zero slope and the mass trend of the slope suggest that we are not in a region of constant k across the mass distribution for all concentrations**
- **Therefore…**

Signal Axis Calibration Model: Use a Taylor's Expansion of R on mass

$$S_i = k_i n_i$$

- **Taylor's expansion of k_i around some M_o, a mass at the center of the distribution**

$$S_i = k_o n_i + Q(m_i - M_o) n_i + \mathrm{O}^2(n_i, m_i) + \ldots$$

- **Q and k_0 are functions of M_0 as well as all of the instrument parameters, the sample concentrations, and the sample preparation method**

Basic form of the Taylor's expansion:

$$f(x) = f(a) + f'(a)(x-a) + \frac{f''(a)}{2!}(x-a)^2 + \frac{f^{(3)}(a)}{3!}(x-a)^3 + \ldots + \frac{f^{(n)}(a)}{n!}(x-a)^n + \ldots.$$

Signal Axis Calibration Model: Seeing What Already We Know

Recall $M_n^{exp} = \Sigma m_i S_i / \Sigma S_i$ & true $M_n^0 = \Sigma m_i n_i / \Sigma n_i$

Substitute S_i from previous slide and after some algebra:

$$M_n^{\exp} = M_n^o \left[\frac{(1 + Q/k_o (M_w^o - M_o)}{(1 + Q/k_o (M_n^o - M_o)} \right]$$

- **Finds true M_n^0 from M_n^{exp}**
- **M_n^{exp} is close to M_n^0 if the polydispersity is "narrow"**
- **Assumes Q/k_o is small**
- **Choose $M_0 = M_n^0$ then above equation explicitly depends on polydispersity**
  - **G. Montaudo (1996)**
- **Need to estimate Q/k_o for each experimental parameter to arrive at uncertainty budget**
- **True of higher order mass moments as well**

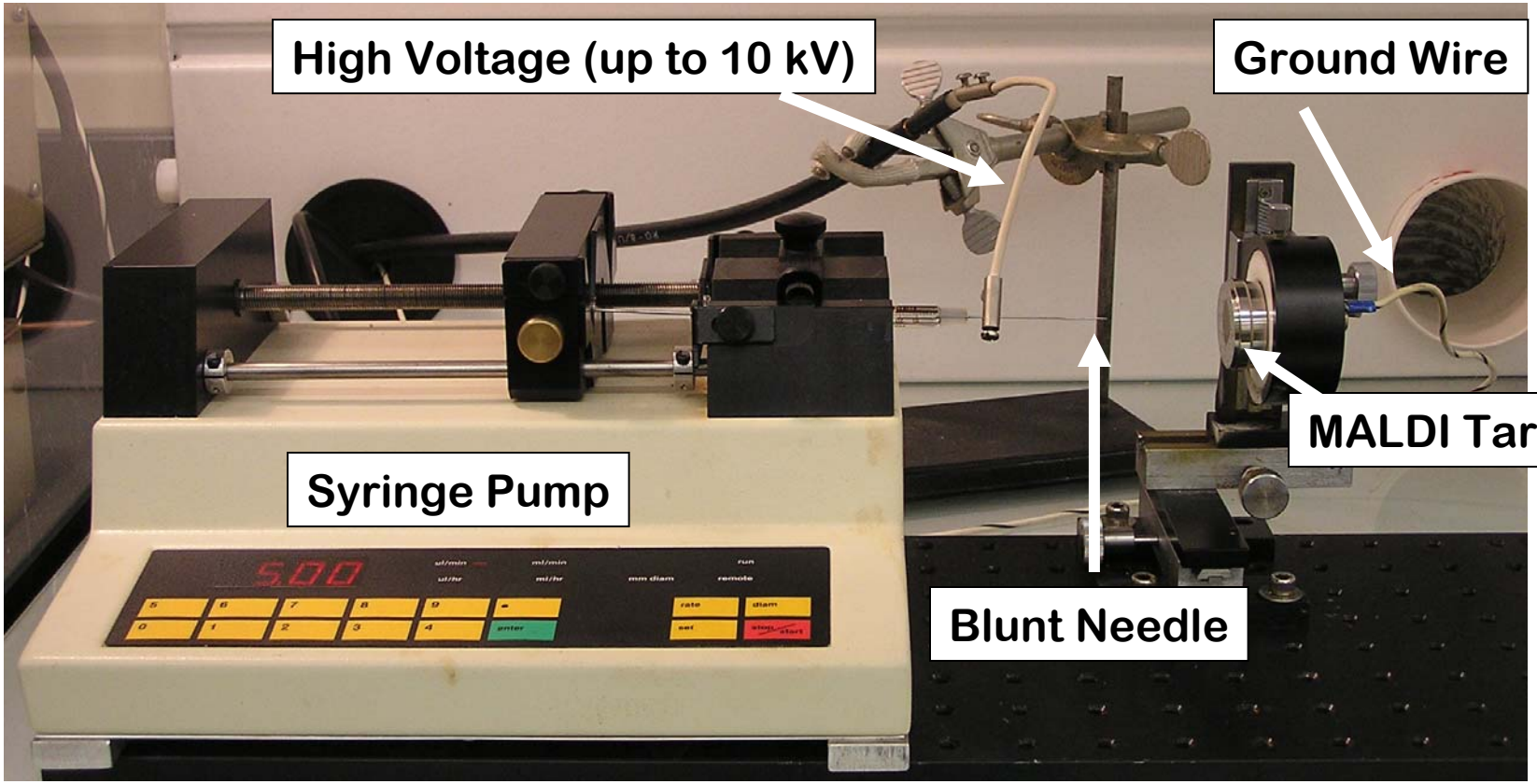
Steps in Creating the SRM

1) Develop sample preparation methods
    - That are repeatable
    - That are fully describable
    - That show little (or no) molecular mass bias, i.e. Q/k_0 small
2) Optimize instrument operating parameters to reduce mass bias
    - Measurement stability a must
3) Develop data analysis methods
    - Operator independent
    - Must be able to handle a wide range of signal to noise
4) Create a calibration curve, i.e. find Q/k_0
    - Transforms mass spectrum into MMD
5) Determine the uncertainty in the calibration curve. i.e. in Q/k_0
    - Types A and B

Step 1: Robust Sample Preparation

- Sample preparation must yield samples that are reproducible over time
  - Day to day, month to month, sample after sample
- A careful experimental procedure was rigorously followed
  - Example: targets, syringes, etc. must be cleaned carefully to prevent cross contamination that leads to false concentration values
- Matrix: *all trans* retinoic acid
- Cationizing agent: silver trifluoroacetate
- Solvent: tetrahydrofuran (unstabilized, but tested for peroxides frequently)
- Mass fractions of analyte:matrx:salt were from NIST interlaboratory comparison (*Anal. Chem.* 73 (2001) 1252)
- Sample composition placed us (roughly) in the center of the linear region of the concentration vs. signal intensity curve as will be shown

Electrospray MALDI Sample Preparation

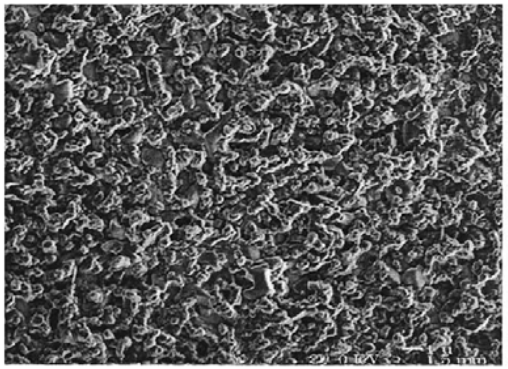


Optical image

1 mm

Operating values:

- 5 μL/min
- 5 kV voltage
- 0.5 mm ID blunt-cut needle
- Spray distance 2 cm
- Spray duration 5 min

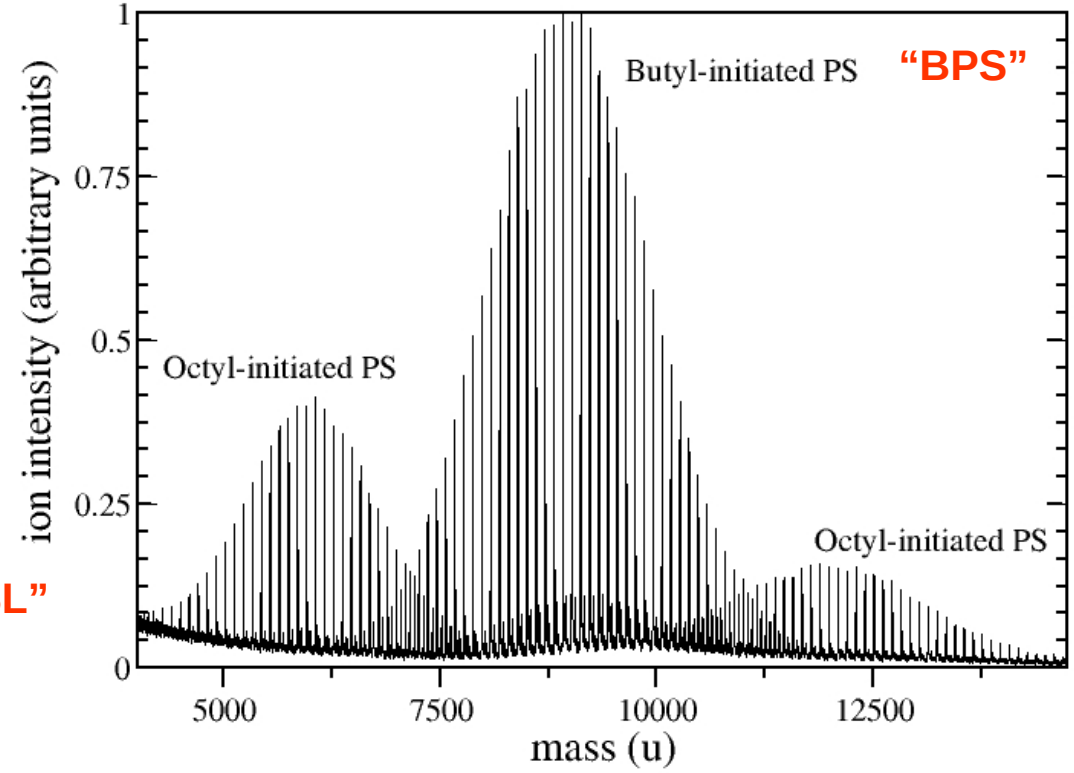


SEM image

1 μm

Step 2: Optimization of Instrument Operating Parameters

- **Goal: Optimize instrument to give most-uniform response across a mixture of three narrow polydispersity polystyrenes, i.e. Q/k_0 as small as possible**
- **Polydispersities of ~1.01**
- **Initiated with n-octyl (6 ku and 12 ku) or n-butyl (9 ku)**
- **Butyl and octyl end groups are inert; octyl group allows for peak separation**
- **Examined by NMR, FTIR, and GPC for purity, MM, and end-group composition**
- **Mixed in a gravimetric ratio of 10:70:20; equal mole ratio for octyl-PS samples**



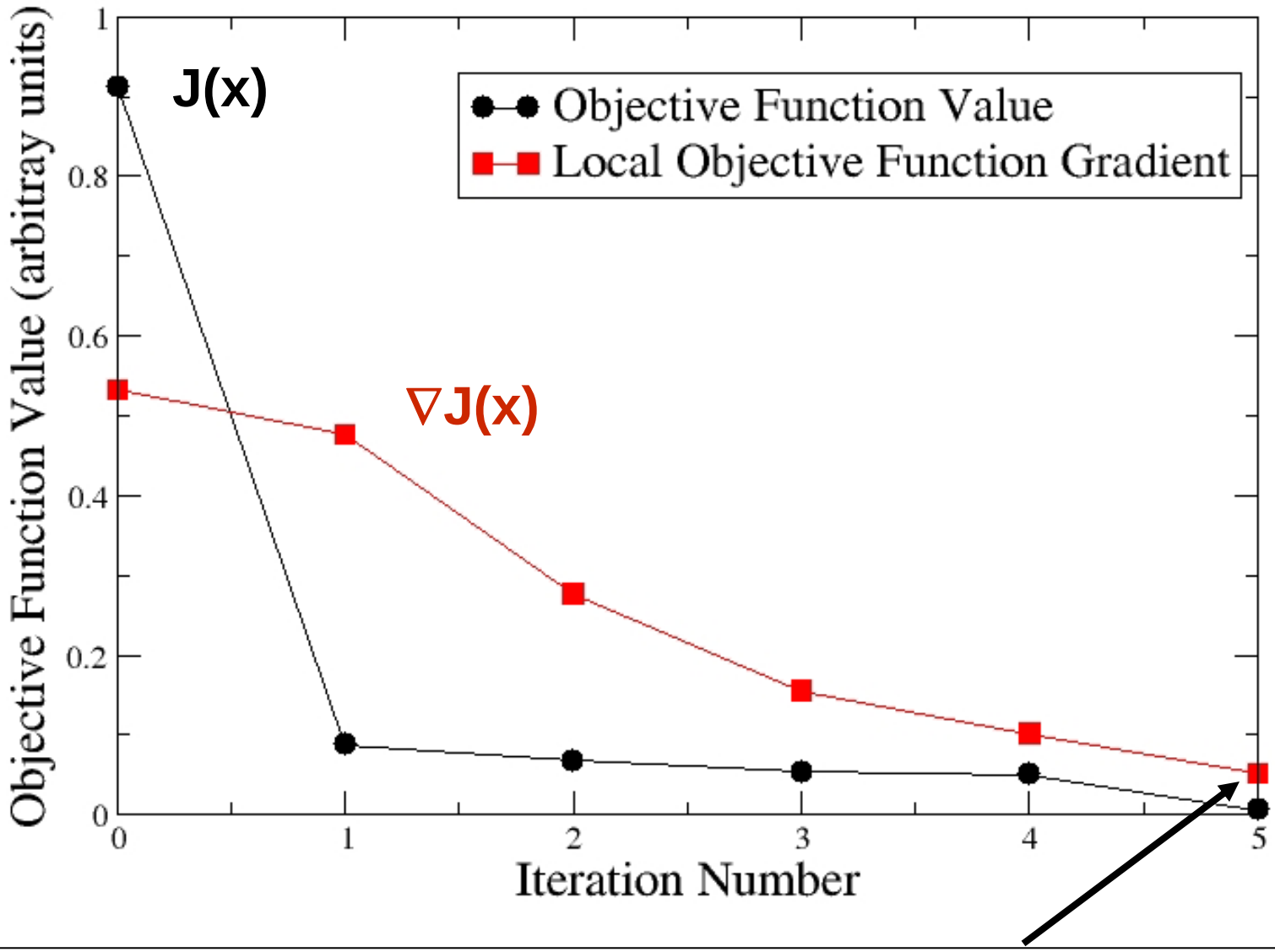
Numerical Optimization of Instrument Settings

- **Optimize 5 adjustable instrument settings**
  - **Extraction, lens, and detector voltages, laser power, delay time**
- **Define an *objective function* J(x)**

$$J(x)=\left(\left(\frac{\sum_{MS} OPSL}{\sum_{MS} BPS}\right)-\left(\frac{OPSL_G}{BPS_G}\right)\right)^2+\left(\left(\frac{\sum_{MS} OPSH}{\sum_{MS} BPS}\right)-\left(\frac{OPSH_G}{BPS_G}\right)\right)^2$$

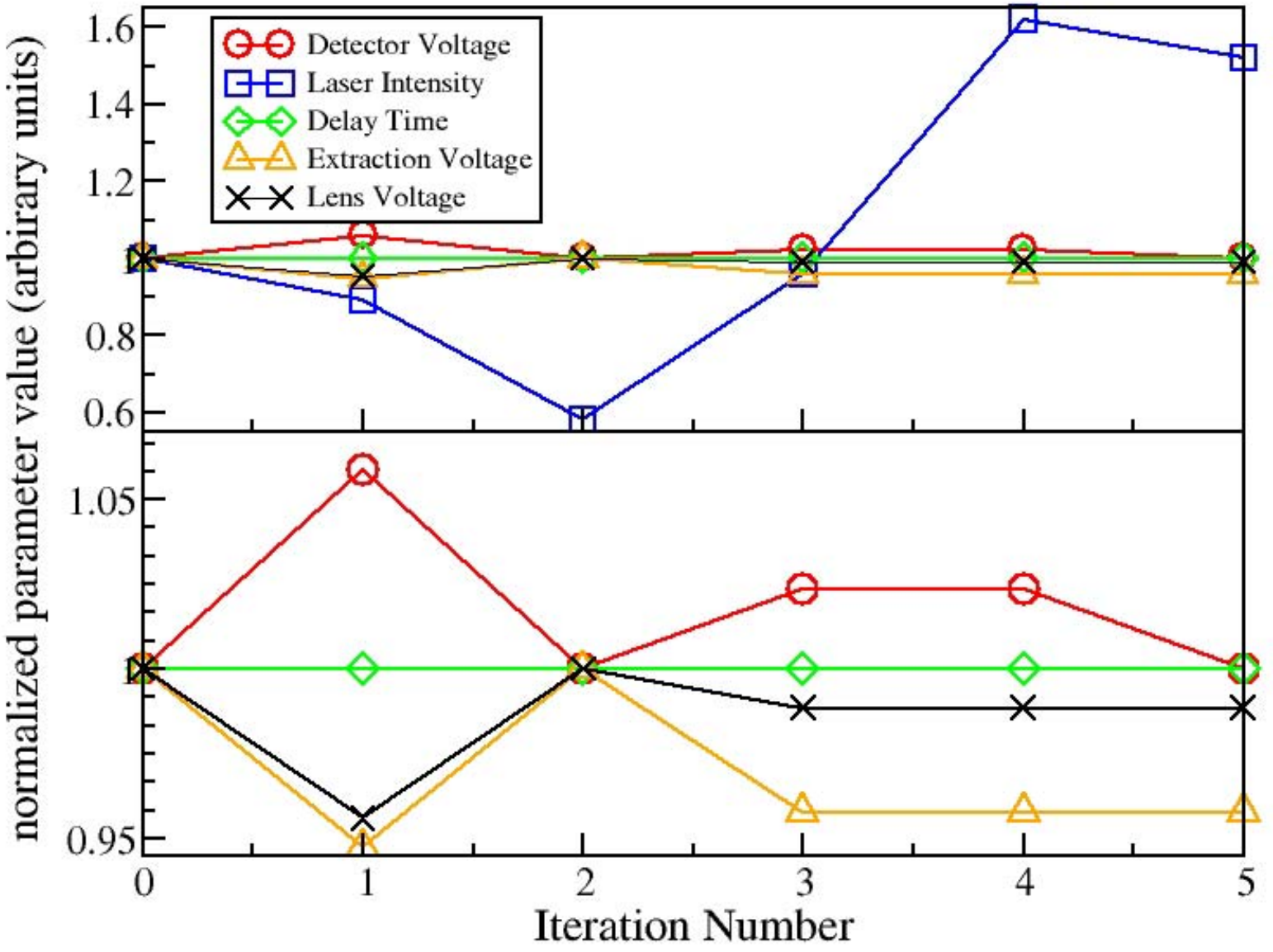
- **Minimize J(x) using stochastic gradient approximations**
  - **Perturb each variable individually**
  - **Calculate local gradient**
  - **Move down gradient**
- **Use random uncertainty in the spectra as a weighting factor**
  - **Using 5 repeats for each change of parameter**

Numerical Optimization of Instrument Settings



Curvature at minimum key to *systematic* uncertainties for each variable

Numerical Optimization of Instrument Settings



Instrument Setting Uncertainties

Calculated at the 95% confidence level

Instrument Parameter	Optimal Setting +/- Confidence Interval
Detector Voltage	1.7 +/- 0.03 kV
Laser Intensity	1.86 +/- 0.11 μJ/pulse
Delay Time	500 ns
Extraction Voltage	18.2 +/- 0.80 kV
Lens Voltage	8.6 +/- 2.0 kV

Sensitive settings have a narrow uncertainty
Less sensitive settings have a relatively wider uncertainty
See: Anal. Chimica Acta 604 (2007) 62-68 for details

Type B Uncertainty from Instrument Setting Uncertainty

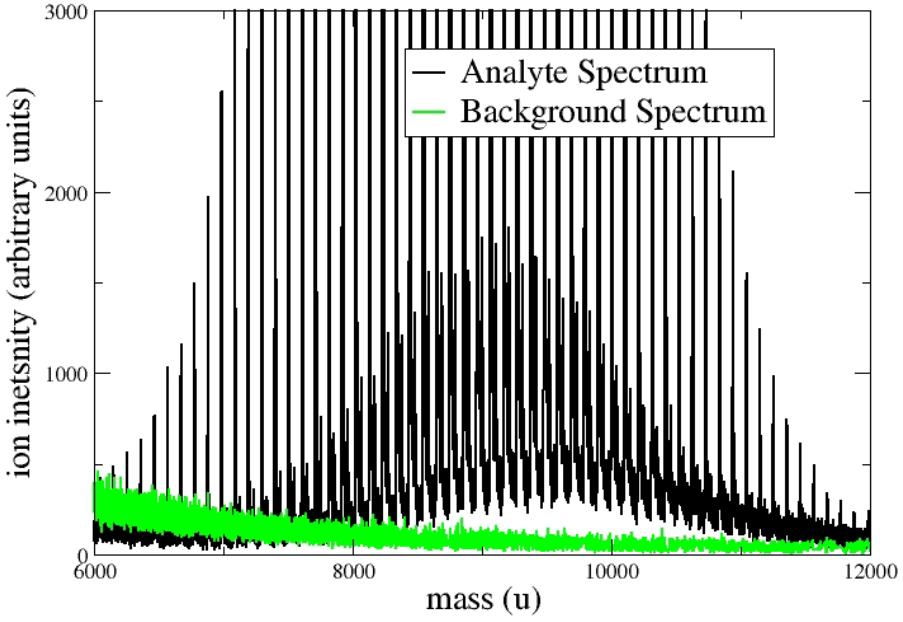
$$J(x) \approx (\mathrm{Q}/\mathrm{k}_0)^2 \{.01(\mathrm{M}_{\mathrm{w}}^{\mathrm{BPSL}} - \mathrm{M}_{\mathrm{w}}^{0})^2 + .04(\mathrm{M}_{\mathrm{w}}^{\mathrm{BPSH}} - \mathrm{M}_{\mathrm{w}}^{0})^2\}$$

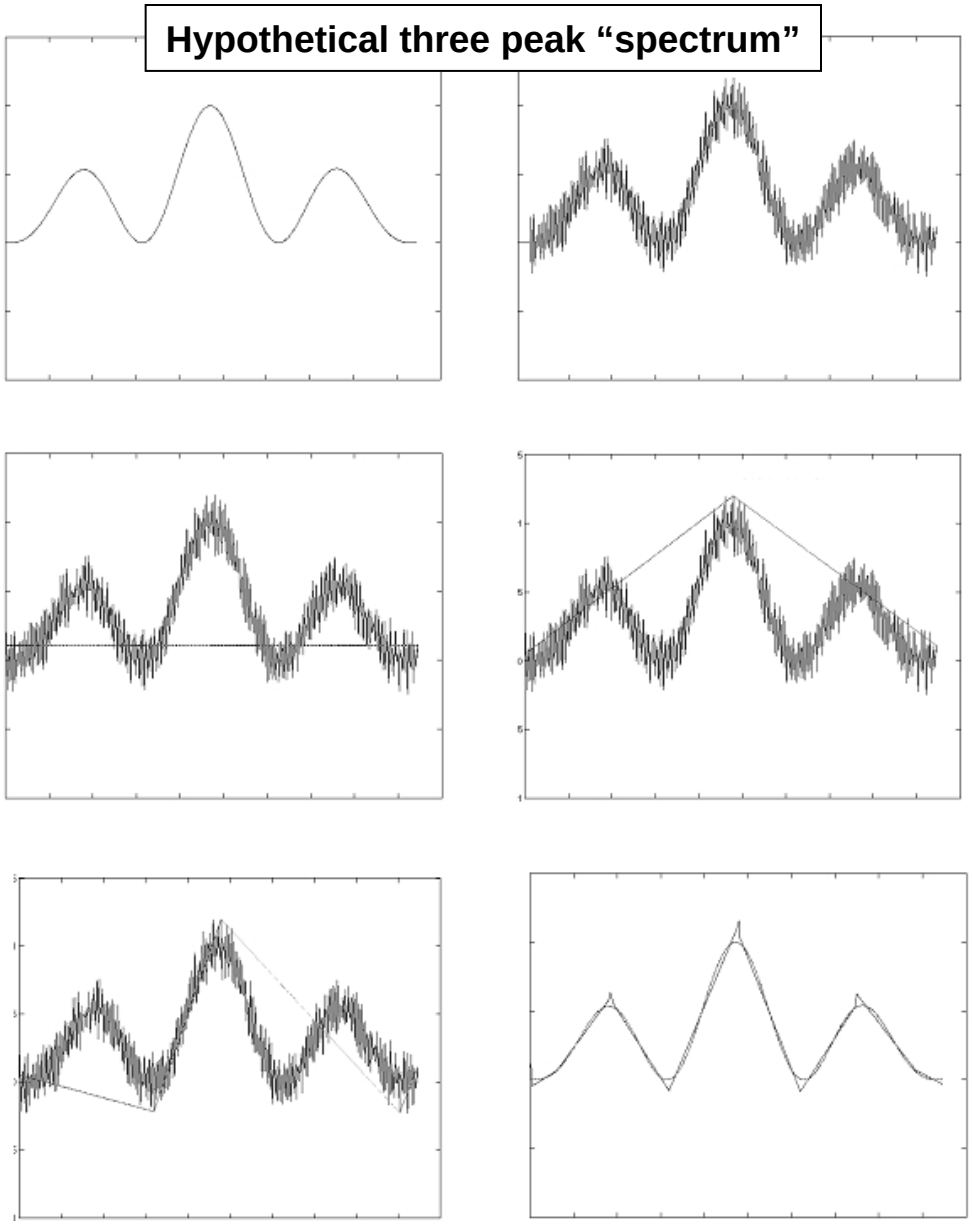
$$\delta \ln(J(x)) / \delta x_i \approx 2\delta \ln(\mathrm{Q}/\mathrm{k}_0)/\delta x_i$$

Instrument Parameter	% Type B Uncertainty Contribution to (B/k_o)
Detector Voltage	0.245%
Laser Intensity	0.15%
Delay Time	—
Extraction Voltage	0.029%
Lens Voltage	0.014%

Step 3: Operator Independent Data Analysis

- ***MassSpectator*** **computer code**
- **Unbiased approach**
- **High throughput, automated**
- **No assumptions on peak shape**
- **Time-series segmentation**
- **Requires a background spectrum**
- ***Anal. Chem.*** **76 (2004) 2446**





Step 4: Calibration Coefficient

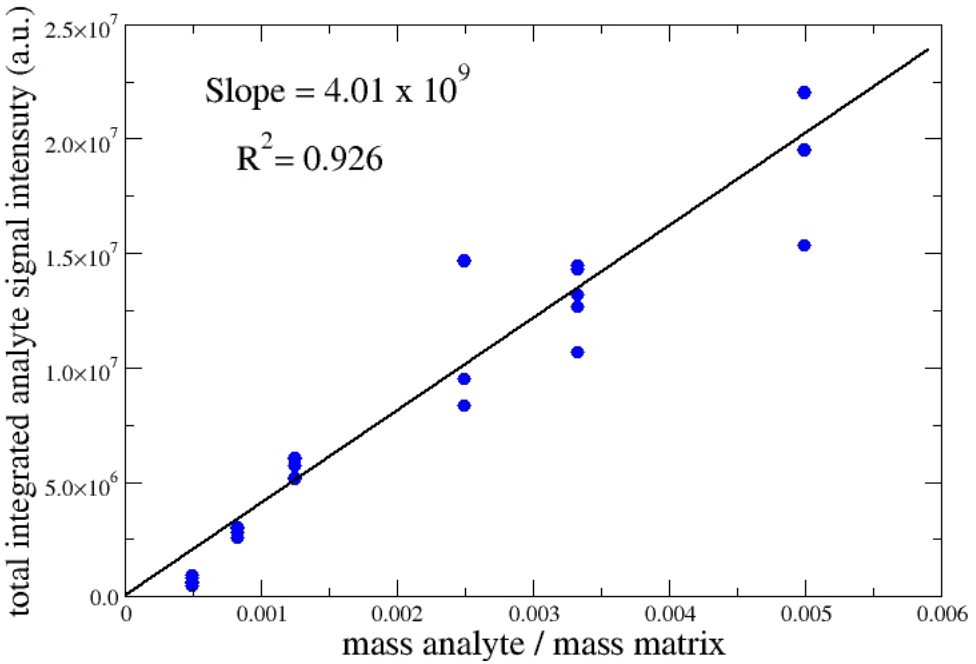
Calibration coefficient relates the signal intensity of oligomer i (S_i) to the relative number of molecules i in the sample (n_i) via the constant k:

$$S_i = kn_i$$

Or, for total amount of signal:

$$\sum S_i m_i = k \sum n_i m_i$$

First you must insure that you are in a region of S-n linearity for small amounts of analyte



Generating the Calibration Curve

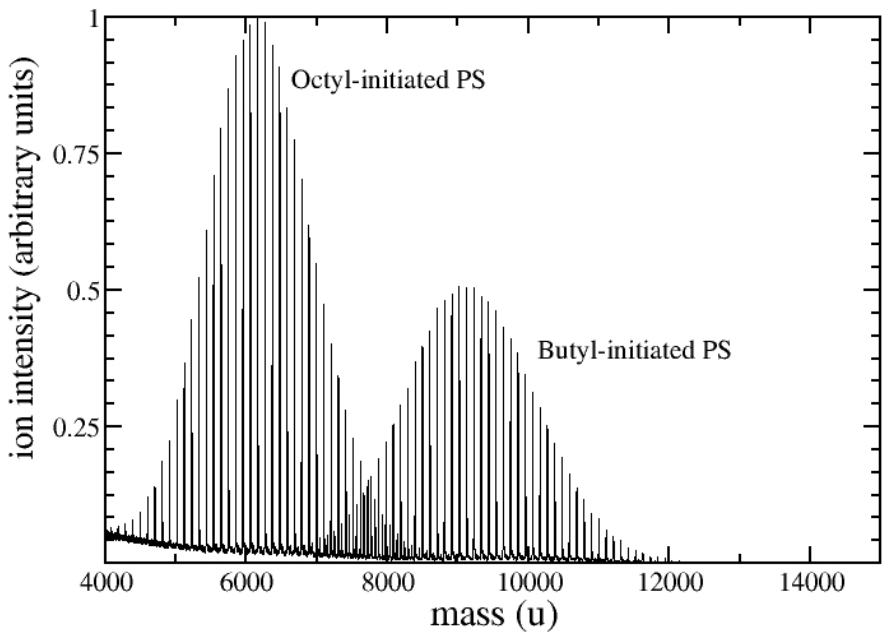
- However, recall that more accurately:

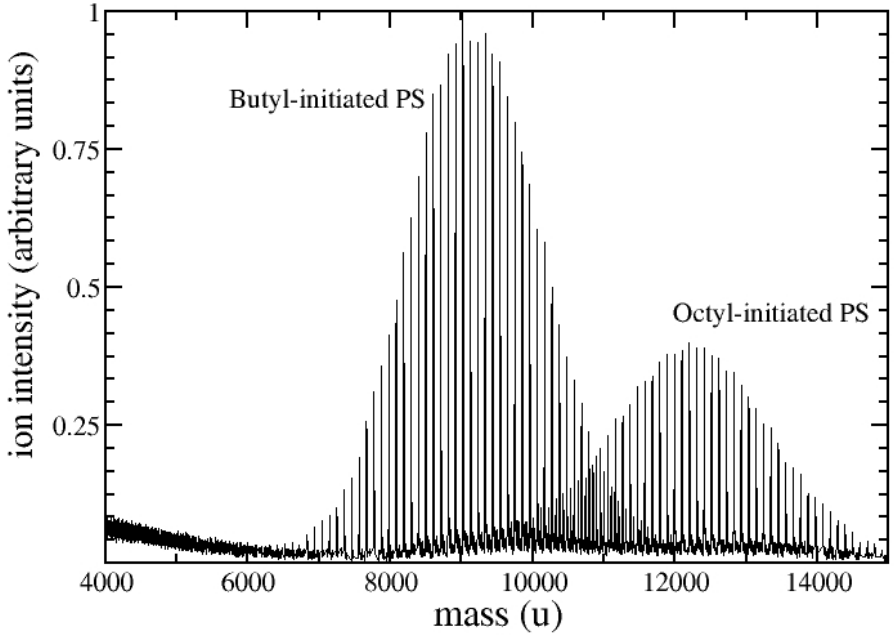
$$S_i = k_i n_i$$

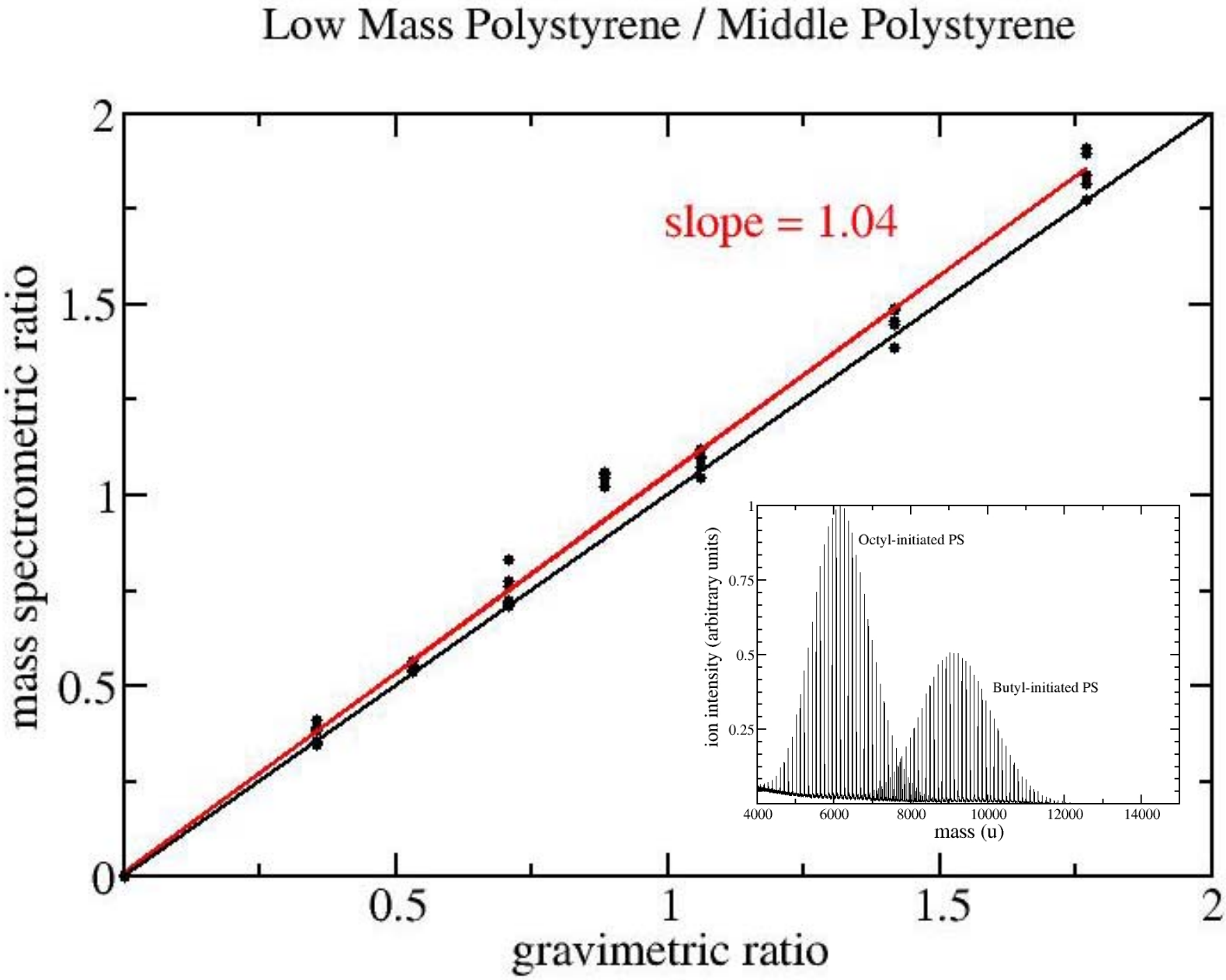
$$S_i = k_o n_i + Q(m_i - M_o) n_i + \mathrm{O}^2(n_i, m_i) + \ldots$$

Generating the Calibration Curve

- **Now systematically vary the Octyl PS/ Butyl PS gravimetric ratios**
- **(For instrument optimization the ratios were fixed)**
- **The gravimetric vs. MS slope will give a data point to calculate Q**
- **Selecting an array of octyl-initiated polystyrenes will give many data points centered at different molecular masses from which the slope Q is calculated**

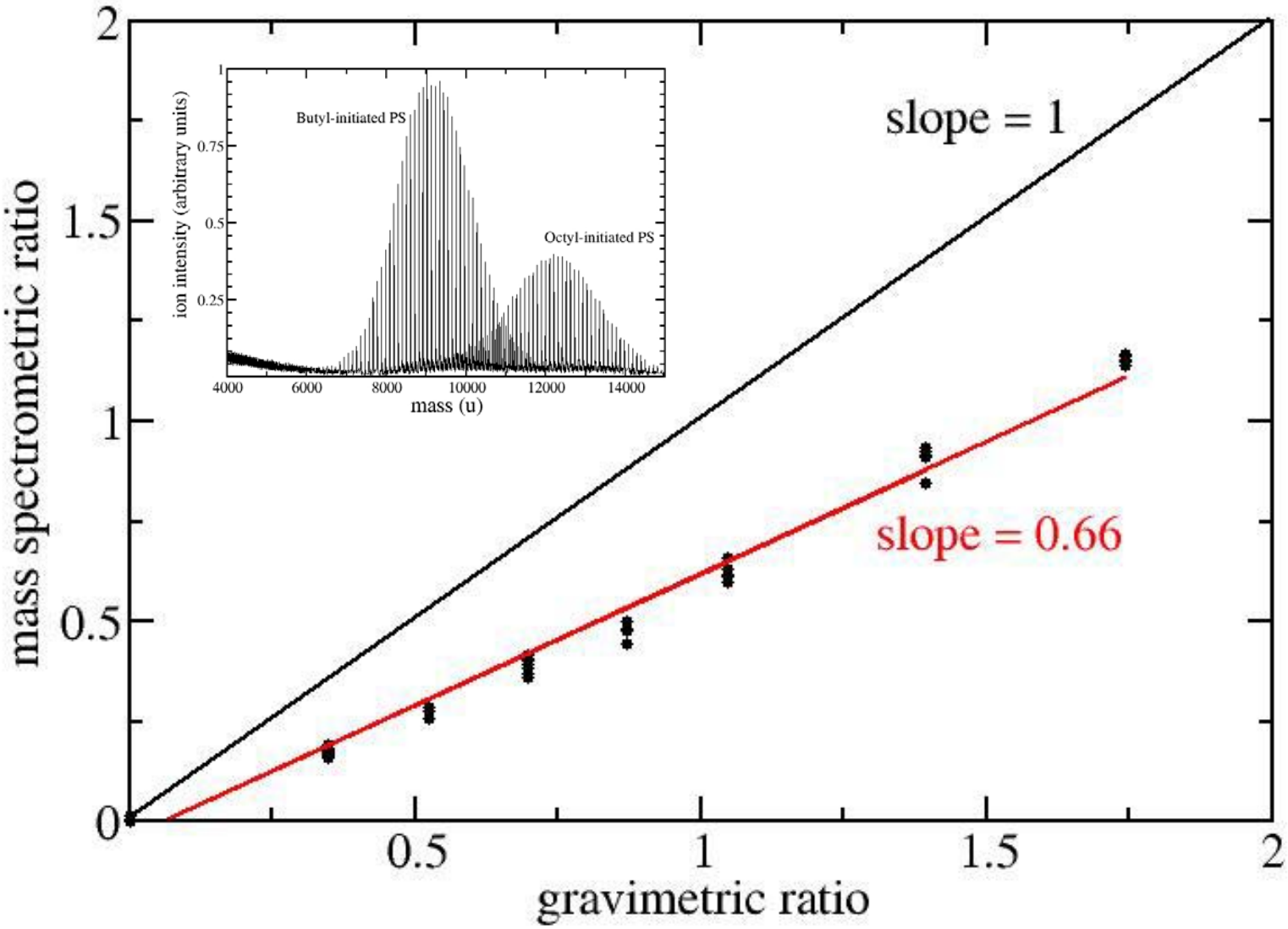






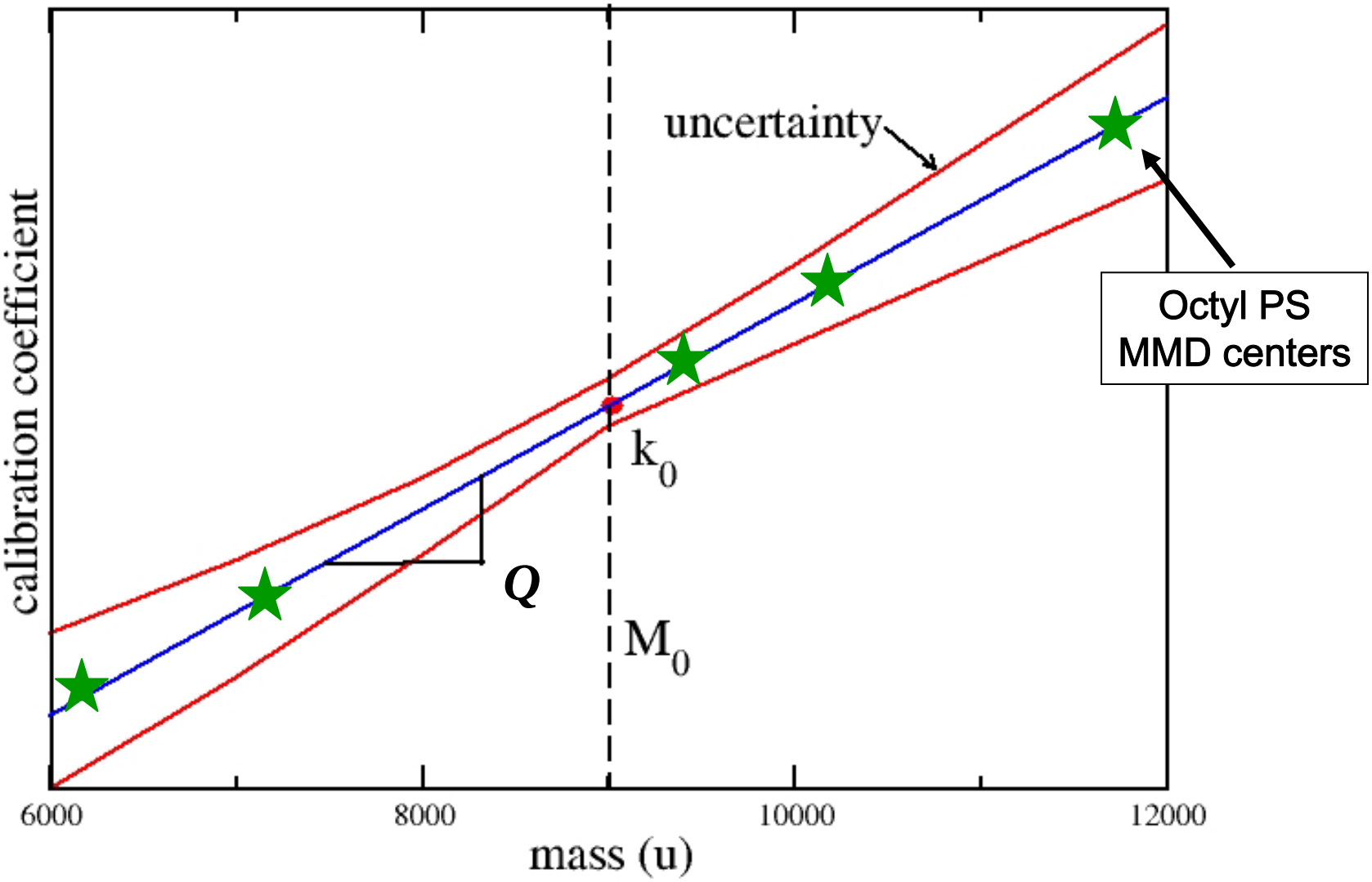
Slope of slightly greater than 1 indicates low mass is over counted

High Mass Polystyrene / Middle Polystyrene



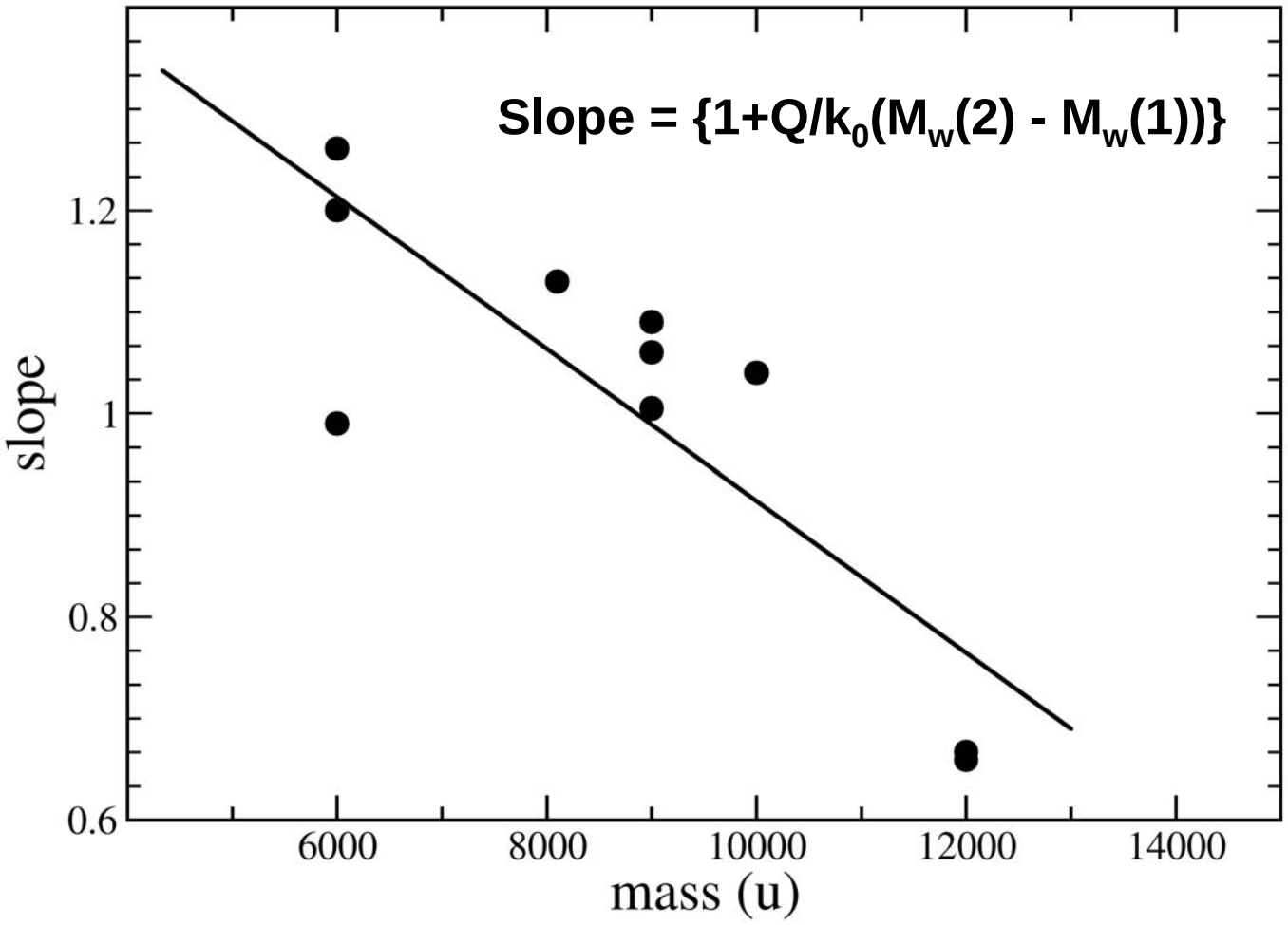
Slope of less than 1 indicates high mass is under counted

Step 5: Uncertainty in the Calibration Curve



$$S_i = k_o n_i + Q(m_i - M_o)n_i + \mathrm{O}^2(n_i, m_i) + \ldots$$

Type A (random) Uncertainty



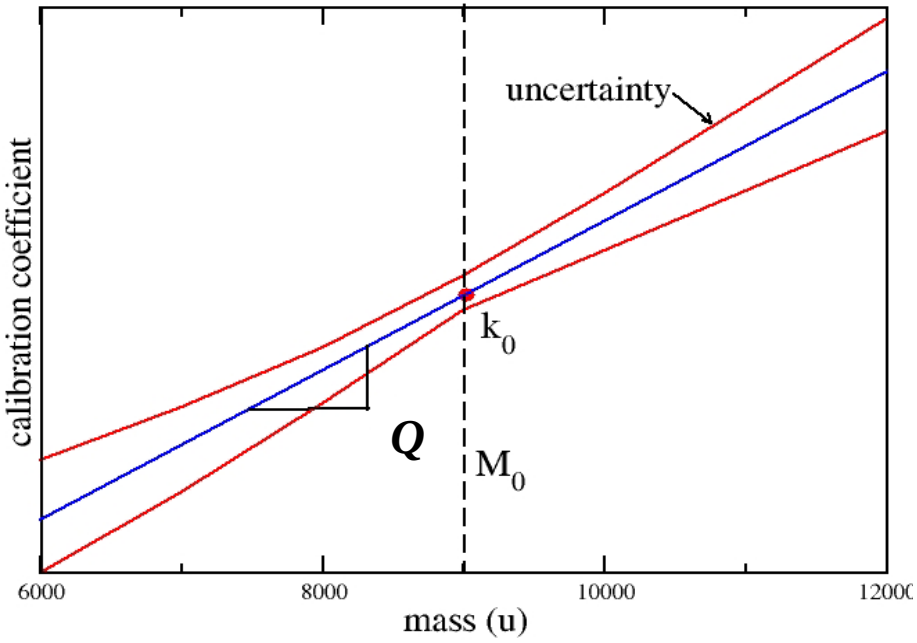
Statistical Uncertainty in Q/k_0 greater than systematic uncertainty !

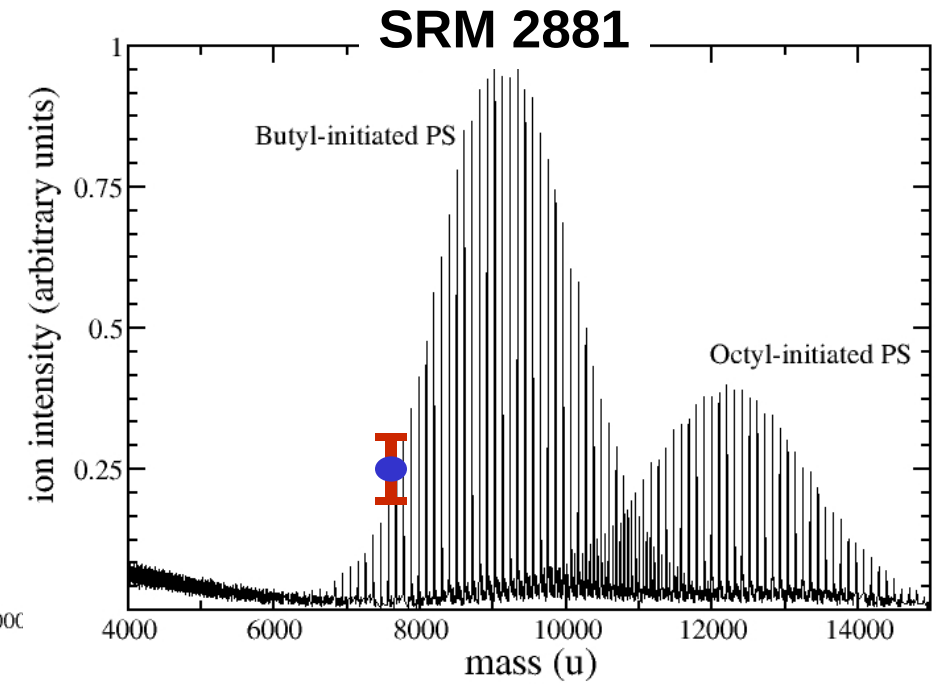
Culmination of All Steps

- **Use the Law of Propagation of Uncertainty**
- **Uncertainty, U, is the square-root of the sum of the squares of the individual contributions (random and systematic)**
- **Systematic uncertainties are weighted by the partial derivative of the function describing the effect of that variable on Q/k_0**

$$U(Q/k_0) = \sqrt{\left(\frac{\partial(Q/k_0)}{\partial x_i}\right)^2 \cdot U_{sys}(x_i)^2 + U_{rand}^2}$$

- **Individual values for the calibration and the uncertainty can be applied to each peak in the distribution**
- **More generally, M_n correction on 9000 u material about 400 u with uncertainty about 200 u**





Summary

- We sought to create an absolute molecular mass distribution standard
- For this we needed type A and type B uncertainties

1) Develop sample preparation methods

2) Optimize instrument operating parameters to reduce mass bias

3) Develop data analysis methods

4) Create a calibration curve

5) Determine the uncertainty in the calibration curve

6) Prepare final SRM certificate